EXHIBIT A

Exhibit A

VOLUME 22 · NUMBER 7 · APRIL 1 2004

ORIGINAL REPORT

Phase II Trial of Cetuximab in Patients With Refractory Colorectal Cancer That Expresses the Epidermal Growth Factor Receptor

Leonard B. Saltz, Neal J. Meropol, Patrick J. Loehrer Sr, Michael N. Needle, Justin Kopit, and Robert J. Mayer

From the Gastrointestinal Oncology Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY; Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA; Indiana University Cancer Center and the Walther Cancer Institute, Indianapolis, IN; Imclone Systems Inc, Somerville, NJ; Bristol Myers Squibb Inc, Wallingford, CT; and the Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA.

Submitted October 24, 2003; accepted December 9, 2003.

Supported by ImClone Systems Inc, Somerville, NJ.

Presented in part at the 38th Annual Meeting of the American Society of Clinical Oncology, Orlando, FL, May 18-21, 2002.

Authors' disclosures of potential conflicts of interest are found at the end of this article.

Address reprint requests to Leonard B. Saltz, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021; e-mail: Saltzl@mskcc.org.

0732-183X/04/2207-1201/$20.00

DOI: 10.1200/JCO.2004.10.182

Purpose
To evaluate the antitumor activity and toxicity of single-agent cetuximab in patients with chemotherapy-refractory colorectal cancer whose tumors express the epidermal growth factor receptor.

Patients and Methods
Phase II, open-label clinical trial. Patients were required to have EGFr expression demonstrated on formalin-fixed paraffin-embedded tumor tissue by immunohistochemical staining before study participation. Patients were required to have received irinotecan, either alone or in a combination regimen, and to have demonstrated clinical failure on this regimen before study entry. Cetuximab was administered weekly by intravenous infusion. The first dose of 400 mg/m² was given during the course of 2 hours. Subsequent weekly treatments were given at a dose of 250 mg/m² during the course of 1 hour.

Results
Fifty-seven eligible patients were treated. All were assessable for toxicity and response. The most commonly encountered grade 3 to 4 adverse events, regardless of relationship to study drug, were an acne-like skin rash, predominantly on the face and upper torso (88% with any grade; 18% with grade 3), and a composite of asthenia, fatigue, malaise, or lethargy (56% with any grade, 9% with grade 3). Two patients (3.5%) experienced grade 3 allergic reactions requiring discontinuation of study treatment. A third patient experienced a grade 3 allergic reaction that resolved, and the patient continued on the study. Neither diarrhea nor neutropenia were dose limiting in any of the 57 patients treated. Five patients (9%; 95% CI, 3% to 19%) achieved a partial response. Twenty-one additional patients had stable disease or minor responses. The median survival in these previously treated patients with chemotherapy-refractory colorectal cancer is 6.4 months.

Conclusion
Cetuximab on this once-weekly schedule has modest activity and is well-tolerated as a single agent in patients with chemotherapy-refractory colorectal cancer whose tumors express the epidermal growth factor receptor. Further studies of cetuximab will evaluate the use of cetuximab in conjunction with first-line and adjuvant treatments for this disease.

J Clin Oncol 22:1201-1209. © 2004 by American Society of Clinical Oncology

INTRODUCTION

Colorectal cancer is a major cause of morbidity and mortality worldwide. In the United States, it is the fourth most common type of cancer and is the second leading cause of cancer death.[1] While early stage colorectal cancer is frequently curable with surgery, unresectable metastatic disease is uniformly fatal. Palliative treatment for metastatic colorectal cancer centers on the use of chemotherapy with the antineoplastic agents fluorouracil (FU), irinotecan, and more recently in the United States, oxaliplatin.[2-5] Once a patient's cancer becomes refractory to these agents, however, there are essentially no established treatment options with demonstrated efficacy. Clearly, there is a desperate need for new and improved therapies for this lethal disease.

Aberrations in the regulation and expression of growth factors and/or their receptors have been extensively implicated in the development and prognosis of malignancies.[6] The epidermal growth factor receptor (EGFr) is a commonly expressed 170,000-kDa *trans*-membrane glycoprotein that is involved in signaling pathways affecting cellular growth, differentiation, and proliferation.[7,8] It is a member of the HER tyrosine kinase growth factor receptor family and is expressed in many types of normal tissues. EGFr expression has been previously demonstrated in multiple tumor types, including colorectal cancer. EGFr is encoded by the proto-oncogene *c-erb-B*.[9,10] Activation of the *c-erb-B* proto-oncogene results in expression of EGFr in many human tumors; hence the initial interest in investigating this growth factor receptor as a potential target for anticancer therapy.

The EGFr is composed of an extracellular ligand-binding region, a lipophilic *trans*-membrane anchor sequence, and an intracellular domain characterized by an ATP-dependent tyrosine kinase.[7,8,11] The extracellular domain of the EGFr is the ligand-binding site for both the epidermal growth factor and for transforming growth factor–alpha (TGF-α). On ligand binding, receptor dimerization occurs, and the tyrosine kinase of the intracellular domain is activated, thereby triggering many signaling reactions that regulate cell growth and survival. Some cells overexpressing the EGFr also produce an endogenous ligand for these receptors, and are thus able to activate the EGFr through an autocrine pathway, which leads to an auto-stimulated proliferation of cell growth and survival in vitro.[12,13] In the laboratory setting, monoclonal antibodies to EGFr are able to inhibit the proliferation of cells that simultaneously produce both EGFr and an activating ligand.[14,15]

Cetuximab (C225, Erbitux™) is an Immunoglobulin G1 human-murine chimeric counterpart of the murine monoclonal antibody M225. Both M225 and cetuximab are antibodies directed against the ligand-binding site of the EGFr.[16,17] M225 has been shown to be able to block the activation of the EGFr tyrosine kinase by EGF or TGF-α, and to inhibit the growth of tumor cells expressing high levels of EGFr.[16-20] Studies have demonstrated that both M225 and cetuximab exhibit similar capacities for competing with ^{125}Iodine-labeled EGF for binding to the EGFr.[17] Furthermore, cetuximab has shown similar growth-inhibitory capacity to M225 in cell culture assays.

Studies in cell lines have shown the capacity of MAb 225 (either M225 or cetuximab) to modulate tumor cell proliferation, cell cycle phase distribution, apoptosis, and radiosensitivity.[21] Mechanisms of anti-EGFr antibody blockade leading to cell cycle arrest have been extensively investigated. Blockade of the EGFr with a monoclonal antibody in the DiFi colon cancer cell line, an autostimulating cell line that is dependent on autocrine EGFr stimulation for survival, induces a cell cycle arrest in G_1. Further studies exploring the mechanism of EGFr blockade-induced G_1 arrest in the DiFi colorectal carcinoma cell line demonstrate that addition of the M225 to cell cultures in saturating concentrations resulted in substantial CDK2 kinase inhibition and Rb hypophosphorylation.[22,23] CDK2, cydin E, and cyclin A protein levels were unaffected; however, protein levels of the CDK inhibitor p27KIP1 were found to be increased two- to three-fold, and removal of p27KIP1 from cell extracts restored activity of CDK2. Other cell lines were also subsequently shown to have G_1 arrest associated with elevated levels of p27KIP1 following exposure to MAb 225.[24,25]

While the vast majority of preclinical data with cetuximab alone has demonstrated primarily cytostatic activity, data combining cetuximab with marginally effective or ineffective cytotoxic chemotherapy have demonstrated marked synergy with dramatic improvement in antitumor activity for the combination.[26-28] Two consistent findings in these preclinical studies are that EGFr expression is needed for cetuximab activity, and that cetuximab, while having modest activity as a single-agent in some systems, has consistently better activity when given in conjunction with inactive or minimally active doses of cytotoxic chemotherapy. Recent studies of cetuximab plus irinotecan in irinotecan-resistant human colorectal tumor xenografts have shown marked synergy between these two agents, with antitumor activity seen with the combination of cetuximab plus irinotecan, while tumor growth occurred with either agent alone.[29]

Based on this scientific background, and encouraged by a favorable anecdotal case of a patient with irinotecan-refractory colorectal cancer responding to cetuximab plus irinotecan,[30] we conducted a phase II study to formally evaluate the activity and safety of cetuximab plus irinotecan in patients with irinotecan-refractory colorectal cancer. That trial was found by an independent response assessment review committee to show a 22.5% major objective response rate in a population of 120 colorectal cancer patients who were determined by their treating physicians to have previously experienced clinical failure on irinotecan.[31] This positive finding for the combination prompted the clinical and regulatory need to define the activity, or lack thereof, of single-agent cetuximab in a similar patient population. We therefore conducted a phase II trial to assess the safety and efficacy of single agent cetuximab in patients with chemotherapy-refractory metastatic colorectal cancers that express EGFr.

PATIENTS AND METHODS

Study Design and Entry Criteria

We conducted a phase II, nonrandomized, open-label, multicenter trial. To be eligible, patients had to have histologically or pathologically documented colorectal cancer and measurable metastatic disease. In addition, immunohistochemical evidence of EGFr expression measured semiquantitatively (> 0 on a scale of 0, 1+, 2+, or 3+) in a single reference laboratory was required.

These measurements were performed and graded using a now commercially available kit (EGFRpharmDx; Dako Corporation, Carpentino, CA) according to the manufacturer's instructions. Patients were permitted to undergo the screening process for tumor EGFr expression before meeting other entry criteria and before study entry. For example, patients were allowed to undergo EGFr screening before study registration, while still receiving initial irinotecan therapy, before documentation of clinical progression. However, to be included in this study, patients were required before study entry to have demonstrated radiologic evidence of failure, as determined by the treating physician, on irinotecan or an irinotecan-containing regimen. Patients were not permitted to have received additional chemotherapy between the time of documented irinotecan failure and entry onto this clinical trial. Patients were required to have at study entry an Eastern Cooperative Oncology Group performance score of 0 to 2, and were required to have an absolute neutrophil count of ≥ 1,500 cells/mm^3, a platelet count of ≥ 100,000 cells/mm^3, a WBC count ≥ 3,500 cells/mm^3, and a hemoglobin level of ≥ 9 g/dL. The total serum bilirubin level was required to be ≤ 1.5× the upper limit of normal, and the alkaline phosphatase and AST levels were required to be ≤ 5× the upper limit of normal. The serum creatinine level was required to be less than 1.5× the upper limit of normal. Patients were required to have recovered from toxicities of prior chemotherapy, and may not have had either radiation therapy or investigational drug therapy within 4 weeks of initiating cetuximab. Bidimensionally measurable disease outside of any previously irradiated field, with a baseline tumor measurement within 4 weeks of initiation of treatment, was required for protocol entry.

This protocol was reviewed and approved by the institutional review board of each participating center, and all patients gave written informed consent before participation.

Therapy

Patients were scheduled to receive cetuximab once weekly. On day 1 of treatment, an initial dose of 400 mg/m^2 was given by a 2-hour intravenous infusion. This loading dose was preceded by a 20-mg test dose to observe for evidence of allergic reactions. All patients were to be premedicated with diphenhydramine 50 mg intravenously. No routine antiemetic medications were given. Cetuximab infusions were then continued weekly at a dose of 250 mg/m^2 unless toxicity necessitated interruptions. The dose and schedule used in this phase II trial were selected to be consistent with those used in other phase II trials of this agent, particularly in colorectal cancer,[21] and are based on extrapolations from previously derived phase I clinical and pharmacokinetic data.[22]

Evaluation of Patients

Response was assessed every 6 weeks during the course of this study. An objective response was identified as a reduction of at least 50% in the area of all measurable lesions on computed tomography or other scans. All objective responses were required to be confirmed by a follow-up scan at least 4 weeks following documentation of the response. Tumor progression on study was defined as an increase of at least 25% in the overall area of the tumor, or the appearance of new lesions. Response to cetuximab was also evaluated retrospectively by an independent response assessment committee that was blinded to the investigator-reported measurements and assessments. Patients underwent weekly blood counts, and physical examinations were performed at every third week while on study.

Statistical Analysis

The study utilized a modified Gehan two-stage design to allow for early stopping in the event of lack of efficacy. The planned sample size for the study was 40 patients, with 20 in each stage. Ultimately, 57 patients were registered and treated. The higher number was due to more rapid accrual than originally anticipated. Continuation to the second stage of the study was contingent on one or more patients from the first stage having a response (partial or complete). Twenty patients were chosen for the first stage in order to limit to 5% the probability of stopping the study early if the true response rate were 15%.

The primary end point in this study was response rate. An exact, two-sided, 95% CI for response rate was computed using the Clopper and Pearson method.[23] Response as a function of EGFR status was evaluated by Fisher's exact test.

Secondary end points included duration of response, time to progression, survival duration, and toxicity. Toxicities, which were categorized using Coding Symbols for Thesaurus of Adverse Reaction Terms and graded using National Cancer Institute common toxicity criteria version 2.0, were summarized by frequencies and percents. Duration of response, time to progression, and survival duration were estimated using the Kaplan-Meier method[24] and summarized by medians. Kaplan-Meier estimates of survival were generated for patients with no skin rash and those who suffered any grade skin rash during the therapy and the curves were compared using the log-rank χ^2 test.

RESULTS

Patient Characteristics

Tumor samples from 140 patients were centrally screened for EGFr expression for possible registration onto this trial. Of these, 105 patients, or 75%, demonstrated at least a 1+ expression of EGFr. Of these 105 EGFr+ patients, 61 who were determined by their treating physician to have had radiological evidence of failure on irinotecan or an irinotecan-containing regimen and who met all other eligibility criteria were registered on this trial between April 17, 2001, and July 12, 2001, and 57 went on to receive cetuximab. Four patients who were registered experienced clinical deterioration before initiation of cetuximab therapy and were therefore not treated. The demographic breakdown of the 57 treated patients is shown in Table 1. The median age of patients participating in this trial was 56 years, with a range of 28 to 80 years. The median Eastern Cooperative Oncology Group performance score was 0, with a range of 0 to 2, and the median time from last prestudy irinotecan dose to initiation of on-study cetuximab therapy was 2.0 months, with a range of 0.5 months to 10.6 months. Forty-seven patients (82%) were treated within 3 months of their last irinotecan dose, seven patients (12%) were treated within 3 to 6 months of their last irinotecan dose, and 3 patients (5%) were treated more than 6 months after their last irinotecan dose.

Sixteen patients had received one prior regimen for their disease before study entry. All 16 of these had received previous concurrent irinotecan + FU + leucovorin. Forty-

Table 1. Patient Characteristics (N = 57)

Characteristic	No. of Patients	%
Age, years		
Median	58	
Range	28-80	
Primary site		
Colon	44	77
Rectum	13	23
Prior chemotherapy (prestudy)		
One prior regimen	16	28
Two or more prior regimens	41	72
Prior oxaliplatin-based regimen	8	14

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Table 2. Efficacy Results (N = 57) per Response Assessment Committee

EGFr Status	Response As a Function of EGFr Status: Responders	%
1+	1/17	6
3+	0/10	0

NOTE. For overall response, partial response was achieved in five patients (9%; 95% CI, 3% to 19%), and stable disease was seen in 21 patients (37%; minimum duration, 12 weeks). In five patients, the median duration of response was 4.2 months.
Abbreviation: EGFr, epidermal growth factor receptor.

one patients had received two or more chemotherapy regimens (including adjuvant regimens, if given) for their disease before study entry. The median duration of irinotecan treatment before participation in this trial for all patients was 16 weeks (range, 1 to 97 weeks).

Although oxaliplatin was not approved for use in the United States during the time that this trial was being conducted, eight patients had received prior oxaliplatin-based treatment before enrolling onto this trial.

Duration of Treatment and Dose Intensity of Cetuximab

The median duration of treatment on study was 6.4 weeks (range, 1 to 67 weeks). Nearly all cetuximab doses administered were given at the full planned dose of 400 mg/m^2 for the loading dose and 250 mg/m^2 for subsequent weekly doses. Sixty-three percent of patients (n = 36) received all planned doses of cetuximab during participation on study. Thirty-two percent of patients (n = 18) missed either one or two planned doses of cetuximab. Less than 1% of patients (n = 1) missed more than two of the planned doses of cetuximab. Two patients (3.5%) received only the test dose of cetuximab.

Efficacy

Response rate. All patients who received any cetuximab on this trial were considered evaluable for efficacy. Of the 57 treated, 6 patients (10.5%; 95% exact CI, 4% to 22%) obtained a partial response based on investigator assessments. Twenty additional patients (35%) had either a minor response (tumor reduction between 25% and 49%) or stable disease (either growth or shrinkage of less than 25%) that lasted for at least 12 weeks from the date of initiation of cetuximab treatment. The independent response assessment committee concurred with five of the six investigator-adjudicated responses (major response rate of 8.8%; 95% CI, 3% to 19%) reclassifying one investigator-adjudicated major response as a minor response. As demonstrated in Table 2, response did not seem to correlate with the observed degree of EGFr expression.

In an exploratory analysis of the 16 patients who had received only one prior regimen, only one patient (as determined by both the investigators and the independent review committee) experienced a partial response, for a response rate of 6.3% (95% exact CI, 0.2% to 30.2%). Because of this, we did not see evidence that the response rate was either superior or different in those patients who had received one versus more than one prior regimen; however, the numbers are too small to permit firm conclusions.

Progression-free and overall survival. The median time to tumor progression on this trial was 1.4 months, with a median duration of response of 4.2 months in the five patients judged by both the investigators and the independent response assessment committee to be major responders. The median survival from time of initiation of protocol therapy for the 57 treated patients was 6.4 months.

Adverse Events

Skin reactions were observed in 88% of patients. In 86% of patients, we saw a subset of skin reaction, acne-like rash, which is characteristic of cetuximab toxicity, as reported previously.[3] Eighteen percent (n = 10) were grade 3. No grade 4 skin reactions were observed. This rash characteristically manifested within the first 1 to 3 weeks of therapy. Standard topical administration of drying agents,

Table 3. Grade 3 or 4 C225-Related Adverse Events

Adverse Event	Patients (N = 57) No.	%
Allergic reaction	3	5
Atrial fibrillation	1	2
Diarrhea	1	2
Hypokalemia	1	2
Vomiting	1	2

topical antibiotics, or topical steroids did not seem to substantially affect the course of the rash, though in patients who appeared clinically to have a bacterial superinfection of the rash, oral antibiotics seemed to provide some clinical improvement. In general, some degree of spontaneous partial improvement in the rash during the first 1 to 2 months of therapy was subjectively noted in many of patients without modification of the cetuximab dose.

Another manifestation of cetuximab cutaneous toxicity was paronychial cracking. These lesions occurred either on the fingers or toes and were reported in 12% of patients. They tended to be relatively persistent throughout the duration of the patient's treatment. Anecdotally, some individuals required several months to effect complete healing after cessation of therapy of these paronychial lesions.

Two patients (3.5%) experienced grade 3 or 4 allergic reactions leading to cessation of therapy. Both occurred during the test dose, and one of these patients required hospitalization. These reactions were characterized as anaphylactoid in nature, and were managed with epinephrine, antihistamines, corticosteroids, and supportive-care measures, and resolved without sequelae. One additional patient experienced a grade 3 allergic reaction on day 233. The patient continued in the study and subsequently discontinued on day 310 due to disease progression. A listing of all grade 3 or 4 cetuximab-related adverse events is presented in Table 3. No grade 3 or 4 neutropenia or thrombocytopenia was encountered.

Correlation of Survival With Rash

There was a correlation between the presence and severity of the acnelike rash and survival. As presented in Table 4, patients with skin rash of any grade had a superior survival to patients with no skin rash ($P = .02$). There was a trend towards improved survival with increasing grade of rash, with patients with grade 3 rash seeming to have the longest survival, and patients with grade 1 or 2 skin rash having intermediary survival relative to those with grade 3 or grade 0.

DISCUSSION

Treatment options for colorectal cancer patients after failure of FU and irinotecan are extremely limited. At the time that this trial was conducted, there were no approved standard treatments in the United States for patients in this setting. For patients previously treated with bolus FU, a course of infusional FU therapy is often attempted, though there is scant if any literature to support this practice. Recently, capecitabine, an oral FU prodrug with antitumor activity in the first-line treatment of colorectal cancer,[35] has become a popular replacement for infusional FU in the irinotecan-refractory setting, though data indicate a lack of significant efficacy of this agent in this refractory setting. Since the conclusion of our trial, oxaliplatin, a diamino cyclohexane platinum analog has become commercially available in the United States, with approval based on a trial that showed a 9.9% major objective response rate for the combination of oxaliplatin plus infusional FU/leucovorin (FOLFOX-4), and virtually no activity of oxaliplatin as a single agent.[36] From a mechanistic perspective, there is no reason to expect cross-resistance between oxaliplatin and cetuximab, and indeed, some of the responses in our trial were noted in patients who had received prior oxaliplatin-based therapy. Both cetuximab and FOLFOX-4 have modest levels of clinical activity in irinotecan-refractory colorectal cancer, and each would seem to bring some modest potential for clinical benefit to this population of patients who are in desperate need of additional therapeutic alternatives.

Preclinical data predict a substantially higher degree of clinical activity for cetuximab in combination with a cytotoxic agent than would be expected with cetuximab treatment alone. Using the concept of evidence-based, rational

Table 4. Survival in Months, in Relationship to Skin Rash

Skin Rash	Grade 0	Grades 1 and 2	Grade 3	Log-Rank P*
No. of deaths at time of analysis	7	32	7	

*Grade 0 versus grades 1 to 3.

design of clinical trials based on preclinical data, the combination of irinotecan plus cetuximab was explored first.[31] The identification of activity with cetuximab plus irinotecan in a population of patients in whom irinotecan had previously failed, seemed to confirm preclinical expectations. Nevertheless, the activity seen on that trial of cetuximab plus irinotecan raised the question of what single-agent cetuximab might accomplish in this setting. This clinical trial to investigate this question in irinotecan-refractory colorectal cancer patients was therefore performed. Subsequently, Cunningham et al[37] performed a confirmatory randomized phase II trial that evaluated both cetuximab alone and cetuximab plus irinotecan in patients with irinotecan-refractory colorectal cancer. The response rates reported in that trial in abstract form were 10.8% for cetuximab alone and 22.9% for cetuximab plus irinotecan. These numbers very closely approximate the results of the trial we report here, as well as those of the previously reported combination trial,[31] confirming the preclinical hypotheses that cetuximab has modest preclinical activity and the combination of cetuximab plus irinotecan has superior activity in irinotecan-refractory colorectal cancer.

The response rate of 9% in this chemotherapy-refractory group of patients is equivalent to the level of activity seen in a similar refractory colorectal population with the FOLFOX combination of oxaliplatin and biweekly infusional FU + leucovorin, although the progression-free survival of 1.4 months is shorter than that reported for FOLFOX.[36] As such, it seems that single agent cetuximab provides a salvage option that may be comparable, at least in terms of antitumor response, to the degree of efficacy seen with oxaliplatin based-combinations following irinotecan/FU failures. Results reported previously with irinotecan plus cetuximab appear to be superior to both cetuximab alone and to what has been reported for FOLFOX in this setting, though randomized comparisons would of course be required to assess the relative efficacy and tolerability of FOLFOX versus cetuximab or versus cetuximab-based combinations in this setting.

The toxicity of cetuximab on this trial was manageable, and was similar to those previously reported for this agent.[31] These included grade 3 or 4 allergic reactions (5%) characterized by typical symptoms of severe hypersensitivity and rapidly responsive to standard management with epinephrine, antihistamines, and corticosteroids. Also seen was the typical skin reaction associated with this agent, and with other agents that block EGFr signaling. This rash, while at times quite esthetically unpleasing, was rarely a source of physical discomfort. None of the 57 patients discontinued treatment on this trial because of the skin rash.

An exploratory analysis suggests a correlation between the presence and severity of the rash and survival. We can only hypothesize as to the meaning of this at this time. The rash may be an indicator of sensitivity to EGFr blockade, both in the patient and the tumor. One possibility is that the rash is a surrogate indicator of an adequate degree of receptor saturation by cetuximab. If this is the case, then targeting doses to achieve a desired level of cutaneous toxicity may further increase the efficacy of this agent. While this is an appealing prospect from a potential efficacy point of view, it would suggest, if true, that there might be a narrow therapeutic window to work with for this agent. Trials are being planned to test this hypothesis, with those patients who do not experience a skin rash after the first few weeks of therapy receiving dose escalations until cutaneous toxicity occurs. Alternatively, it is possible that a particular EGFr polymorphism might correlate with both antitumor activity and cutaneous toxicity. Further studies of this possibility are also planned.

Preclinical data have suggested synergy between cetuximab and a number of cytotoxic agents, including irinotecan, cisplatin, gemcitabine, and doxorubicin. The exact mechanism of this synergy has not been definitively determined. A working hypothesis is that abrogation of activity in the EGFr-mediated signaling pathways leads to a decrease in EGFr-mediated antiapoptotic messages transmitted within the cell. This may be sufficient for cytotoxicity in some cell lines that sustain an autocrine loop of EGF production and EGFr expression and that are particularly dependent on this pathway for survival. In the majority of cell lines studied, however, the blockage of EGFr signaling is insufficient for cytotoxicity, but may leave the cells more vulnerable to the cytotoxic effects of otherwise minimally efficacious chemotherapy.

The favorable clinical response rate seen in this single agent trial raises a number of important clinical questions, which are the subject of further ongoing and/or planned investigations. The lack of correlation between the degree of EGFr expression and response raises the question of whether non-EGFr–expressing tumors might also be potentially sensitive to cetuximab-based therapy. It would appear, on the basis of these data, that immunohistochemistry for EGFr expression is a poor indicator of which tumors are most treatable by this EGFr-targeted therapy. Other means of assessing EGFr expression, such as RT-PCR, will need to be explored. Correlative studies will also be needed to assess downstream effects of EGFr blockade to further elucidate patterns that may be predictive of cetuximab activity and so permit appropriate selection of patients for treatment.

Based on preclinical data, we would anticipate that the greatest degree of benefit from cetuximab in colorectal cancer is likely to be seen in combination with chemotherapy in first line or adjuvant use, although we cannot know the actual utility of such an approach until data are available from randomized trials. Pilot trials to incorporate cetuximab into first line regimens of irinotecan/FU/leucovorin have been completed,[38] and randomized phase III trials in first line and adjuvant combination use are in the process of activation. Finally, heterogeneity in the qualitative, as well

as quantitative expression of EGFr, may be clinically relevant to the successful application of cetuximab. Human EGFr polymorphisms have been described, and future large-scale trials will attempt to characterize these polymorphisms and correlate them with clinical activity and toxicity. Studies are also underway to characterize molecular phenotypes that predict response to this agent.

Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Owns stock (not including shares held through a public mutual fund): Michael Needle, Imclone; Justin Kopit, Bristol-Myers Squibb. Acted as a consultant within the last 2 years: Neal Meropol, Bristol-Myers Squibb. Performed contract work within the last 2 years: Leonard Saltz, Imclone; Neal Meropol, Imclone, Bristol-Myers Squibb. Served as an officer or member of the Board of a company: Michael Needle, Imclone; Justin Kopit, Bristol-Myers Squibb. Received more than $2,000 a year from a company for either of the last 2 years: Michael Needle, Imclone; Justin Kopit, Bristol-Myers Squibb.

REFERENCES

1. Jemal A, Murray T, Samuels A, et al: Cancer statistics 2003. CA Cancer J Clin 53:5-26, 2003
2. Leichman CG, Fleming TR, Muggia FM, et al: Phase II study of fluorouracil and its modulation in advanced colorectal cancer: A Southwest Oncology Group study. J Clin Oncol 13:1303-1311, 1995
3. Saltz LB, Cox JV, Blanke C, et al: Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med 343:905-914, 2000
4. Cunningham D, Pyrhonen S, James RD, et al: Randomized trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. Lancet 352:1413-1418, 1998
5. de Gramont A, Figer A, Seymour M, et al: Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 18:2938-2947, 2000
6. Aaronson S: Growth factors and cancer. Science 254:1146-1153, 1991
7. Carpenter G: Receptors for epidermal growth factor and other polypeptide mitogens. Annu Rev Biochem 56:881-914, 1987
8. Carpenter G, Cohen S: Epidermal growth factor. J Biol Chem 265:7709-7712, 1990
9. Downward J, Yarden Y, Mayes E, et al: Close similarity of epidermal growth factor receptor and V-erb-B oncogene protein sequences. Nature 307:521-527, 1984
10. Real FX, Rettig WJ, Chesa PG, et al: Expression of epidermal growth factor receptor in human cultured cells and tissues. Cancer Res 48:4728-4731, 1988
11. Yarden Y, Ullrich A: Growth factor receptor tyrosine kinases. Annu Rev Biochem 57:443-478, 1988
12. Di Marco E, Pierce JG, Fleming TP, et al: Autocrine interaction between TGF alpha and the EGF receptor: Quantitative requirements for induction of the malignant phenotype. Oncogene 4:831-838, 1989
13. Van de Vijver MJ, Kumar R, Mendelsohn J: Ligand-induced activation of A431 cell epidermal growth factor receptors occurs primarily by an autocrine pathway that acts upon receptors on the surface rather than intracellularly. J Biol Chem 266:7503-7508, 1991
14. Baselga J, Norton L, Masui H, et al: Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. J Natl Cancer Inst 85:1327-1333, 1993
15. Masui H, Bomalaski S, Hyman J, et al: Treatment with anti-EGF receptor monoclonal antibody causes regression of DiFi human colorectal carcinoma xenografts. Proc Am Assoc Cancer Res 32:394, 1991
16. Sato JD, Kawamoto T, Le AD, et al: Biological effects in vitro of monoclonal antibodies to human epidermal growth factor receptors. Mol Biol Med 1:511-529, 1983
17. Goldstein NI, Prewett M, Zuklys K, et al: Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. Clin Cancer Res 1:1311-1318, 1995
18. Gill GN, Kawamoto T, Cochet C, et al: Monoclonal anti-epidermal growth factor receptor antibodies which are inhibitors of epidermal growth factor binding and antagonists of epidermal growth factor binding and antagonists of epidermal growth factor-stimulated tyrosine protein kinase activity. J Biol Chem 259:7755-7760, 1984
19. Masui H, Kawamoto T, Sato JD, et al: Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies. Cancer Res 44:1002-1007, 1984
20. Vande Woude GF, Schultz N, Zhou R, et al: Cell cycle regulation, oncogenes, and antineoplastic drugs. In: Fortner JG, Rhoads JE (eds). Accomplishments in Cancer Res Foundation. Philadelphia, PA, JB Lippincott Co, 1990, pp 128-143
21. Huang SM, Bock JM, Harari PM: Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. Cancer Res 59:1935-1940, 1999
22. Wu X, Fan Z, Masui H, et al: Apoptosis induced by antiepidermal growth factor receptor monoclonal antibody in a human colorectal carcinoma cell line and its delay by insulin. J Clin Invest 95:1897-1905, 1995
23. Xu X, Rubin M, Fan Z, et al: Involvement of p27KIP1 in G_1 arrest mediated by an epidermal growth factor receptor monoclonal antibody. Oncogene 12:1397-1403, 1996
24. Fan Z, Shang BY, Wu X, et al: Blockade of epidermal growth factor receptor by anti-EGFR monoclonal antibody 225 causes G1 arrest of A431 cells with induction of p27KIP1. Proc Am Assoc Cancer Res 37:10, 1996
25. Peng D, Fan Z, Lu Y, et al: Anti-epidermal growth factor monoclonal antibody 225 upregulates p27KIP1 and induced G1 arrest in prostate cancer cell lineDU145. Cancer Res 56:3666-3669, 1996
26. Fan Z, Baselga J, Masui H, Mendelsohn J: Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus cis-diaminedichloroplatinum on well established A431 cell xenografts. Cancer Res 53:4637-4642, 1993
27. Baselga J, Pfister D, Cooper MR, et al: Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. J Clin Oncol 18:904-914, 2000
28. Ciardiello F, Bianco R, Damiano V, et al: Antitumor activity of sequential treatment with topotecan and anti-epidermal growth factor receptor monoclonal antibody C225. Clin Cancer Res 5:909-916, 1999
29. Prewett M, Hooper A, Bassi R, et al: Enhanced antitumor activity of anti-epidermal growth factor receptor monoclonal antibody IMC-C225 in combination with irinotecan (CPT-11) against human colorectal tumor xenografts. Clin Cancer Res 8:994-1003, 2002
30. Rubin M, Shin D, Pasmantier M, et al: Monoclonal antibody IMC-C225 and anti-epidermal growth factor receptor antibody for patients with EGFr-positive tumors refractory to or in relapse from previous therapeutic regimens. Proc Am Soc Clin Oncol 19:674a, 2000
31. Saltz L, Hochster H, Rubin M, et al: Cetuximab (IMC-C225) plus irinotecan (CPT-11) is active in CPT-11-refractory colorectal cancer (CRC) that expresses epidermal growth factor receptor (EGFR). Proc Am Soc Clin Oncol 20:3a, 2001 (abstr 7)
32. Baselga J, Pfister D, Cooper MR, et al: Phase I studies of antiepidermal growth factor receptor chimeric antibody c225 alone and in combination with cisplatin. J Clin Oncol 18:904-914, 2000
33. Clopper CJ, Pearson ES: The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika 26:404-413, 1934

34. Kaplan EL, Meier P: Non-parametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958

35. Hoff PM, Ansari R, Batist G, et al: Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: Results of a randomized phase III study. J Clin Oncol 19:2282-2292, 2001

36. Rothenberg M, Cox AM, Bigelow RH, et al: Superiority of cisplatin and fluorouracil-leucovorin compared with either therapy alone in patients with progressive colorectal cancer after irinotecan and fluorouracil-leucovorin: Interim results of a phase III trial. J Clin Oncol 21:2059-2069, 2003

37. Cunningham D, Humblet Y, Siena S, et al: Cetuximab (C225) alone or in combination with irinotecan (CPT-11) in patients with epidermal growth factor receptor (EGFR)-positive, irinotecan-refractory metastatic colorectal cancer (MCRC). Proc Am Soc Clin Oncol 22:252a, 2003 (abstr 1012)

38. Rosenberg A, Loehrer PJ, Needle MN, et al: Erbitux (IMC-C225) plus weekly irinotecan (CPT-11), fluorouracil (5FU) and leucovorin (LV) in colorectal cancer (CRC) that expresses the epidermal growth factor receptor (EGFr). Proc Am Soc Clin Oncol 20:135a, 2001 (abstr 536)